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Final Report

NAG8-955

CHARACTERIZATION OF SURFACES

A Ground Based, Basic Science Research Proposal Submitted to

NASA/MARSHALL SPACE FLIGHT CENTER

by

The University of Alabama in Huntsville

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ABSTRACT

Low gravity biotechnology experiments indicate a need to better understand and control a host of liquid-solid interfacial phenomena which reduce the efficiency of bioseparations methods on earth as well as in space. We have improved and utilized polymeric and silane derivatives, developed in association with MSFC, in order to control such phenomena. The objectives of the proposed research have been obtained. They were to improve NASA-patented coatings capable of controlling macromolecular adsorption, electroosmosis, and particle electrophoresis over a wide range of pH, and to further characterize the ability of polymeric coatings to control wall wetting interactions. To date this research has resulted in six publications and four abstracts. It has also aided researchers at MSFC with studies on the electrophoresis of large DNA molecules in free solution. It will continue to enhance NASA's efforts to exploit the space environment to enhance knowledge of phenomena relevant to biotechnology, and obtain bioseparations currently unobtainable on Earth.

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1.1 Overview

Low gravity bioprocessing experiments have revealed that a variety of liquid-solid (L-S) interfacial phenomena, known to be deleterious on Earth also disrupt space biotechnical experiments. These phenomena include macromolecular adsorption, electroosmosis, and wall wetting. They counter advantages offered by the low gravity environment of spacecraft (e. g., reduced convection and sedimentation) and negatively impact NASA-supported research in bioseparations.

New approaches, based on enhanced knowledge and control of interfacial phenomena, are needed to improve bioseparation technologies (on earth and in space). We proposed collaborating with MSFC on studies related to control of interfacial phenomena via polymeric derivatives. The proposed research built upon technology developed by the P. I. from MSFC sponsored flight and ground-based research, and described in two MSFC-related patents. The proposed research aimed to improve this technology and test its application by improving MSFC inhouse research into electrophoresis.

James M. Van Alstine (P.I., UAH) directed the UAH program. He was aided in this task by a series of part-time students (Norman Burns followed by Laine Gundy and Ken Emoto). The Van Alstine laboratory worked closely with the laboratory of Professor J. M. Harris (which synthesized the polymeric derivatives used in the research) . Dr. Van Alstine's group also interacted with scientists in the Biophysics Branch (ES76) at the Marshall Space Flight Center (MSFC) and supported the research of MSFC NASA Investigator Percy H. Rhodes' via supplying coatings to improve the performance of experimental chambers used in capillary electrophoresis. This enhanced the electrokinetic separation of large DNA molecules on the basis of MW. These experiments were also supported by an ongoing collaboration with K. Holmberg, Director of the Surface Chemistry Institute in Stockholm (where Dr. Van Alstine is presently on sabbatical). In addition to accomplishing much research and publishing actively (see below) the grant supplied an opportunity for Dr. Van Alstine to submit a major follow-on proposal to NASA HQ.

1.2 Publications and Specific Achievements

The general objectives were to further develop and characterize polymeric derivatives which control certain liquid-solid (L-S) interactions of biotechnical significance. The UAH group proposed to extend their published and patented, NASA supported, research in three specific areas, 1) coatings to control nonspecific (macromolecular) adsorption, 2) coatings to control electroosmosis, and 3) coatings to control wall wetting.

Specific objectives were:

1. Continue development of long-lasting coatings which control electroosmosis over a broad pH range. Maintenance of promising new research directions related to organosilane sublayers, and poly-(ethyleneimine) sublayers, and polysaccharide coatings.
2. Apply chemical surface modification techniques to support MSFC investigations related to ascertaining molecular mechanism(s) that may support the utility of externally-applied electric fields in capillary zone electrophoresis.
3. Apply chemical surface modification techniques to support MSFC investigations related to the electrokinetic characterization and separation of large DNA molecules.
4. Further develop biphasic contact angle goniometry and chemical surface modification to control phase wall wetting.

Tasks 2 and 3 involved direct interaction with P. Rhodes et al. of MSFC ES76.

The above tasks were accomplished as evidenced by the following publications - copies of which, and whose exact content and nature, have been supplied to MSFC with previous progress reports. Copies of Abstracts related to two recent papers are provided in Appendix I. Appendix II provides the abstract and title page of the NASA HQ proposal that resulted from this work. Appendix III provides a list of papers, abstracts and publications from the research continued under the present grant (NAG8-955).

New Technology

The Langmuir publication by Burns et al (see below) provides a method to electrokinetically evaluate chemical surface groups in a non-destructive manner. The information obtained relates to surface density and chemistry, e. g., pKa, of multiple types of surface groups. For this reason I believe that it should be evaluated as New Technology.

Publications

01. Burns, N.L., Van Alstine, J.M., and Harris, J.M. 1994. Poly(ethylene glycol) Grafted Onto Quartz: Surface Analysis via a Site Dissociation Model of Electroosmosis. Langmuir, submitted. **Abstract Attached**
02. Österberg, E., Bergström, K., Holmberg, K., Schuman, T.P., Riggs, J.A., Burns, N.L., Van Alstine, J.M. and Harris, J.M. 1994. Protein Rejecting Ability of Surface Bound Dextran in End-On and Side-On Configurations. J. Biomaterials, submitted. **Abstract Attached**
03. Österberg, E., Bergström, K., Holmberg, K., Van Alstine, J.M., Riggs, J. A., Schuman, T.P., Burns, N.L., and Harris, J.M. 1993. Comparison of Polysaccharide and Poly(ethylene glycol) Coatings for Reduction of Protein Adsorption on Polystyrene Surfaces. Colloids and Surfaces, 77, 159-169.
04. Van Alstine, J.M., Burns, N.L. , Riggs, J.A., Holmberg, K. and Harris, J.M. 1993. Electrokinetic Characterization of Hydrophilic Polymer Coatings of Biotechnical Significance. Colloids and Surfaces, 77, 149-158.
05. Van Alstine, J.M. Burns N.L., Riggs, J.A., Holmberg, K. and Harris, J.M. 1993. Electrokinetic Characterization of Polysaccharide and Related Coatings. In Carbohydrates and Carbohydrate Polymers, Yalpani, M. (Ed.) ACS Symposia, ATL Press, pp. 298-309.
06. Van Alstine, J. M., Burns, N. L., Atharale, S., Holmberg, K and Harris, J. M. 1993. Use of Micro Particle Electrophoresis to Characterize Hydrophilic Polymer Coatings. American Chemical Society National Meeting, Chicago, IL, August 1993, Published as an Extended 8 Page Abstract in the Proceedings of the ACS PMSE Meeting.

Abstracts

- Ø 1. Harris, J. M., Van Alstine, J. M., Burns, N. L., Riggs, J. A., Shuman, T. P. and Holmberg, K.. 1992. Protein Rejection by Polysaccharide Modified Surfaces. NSF EPSCOR Materials Research Meeting and 6th Annual Alabama Materials Research Conference, Auburn University, Auburn, AL, October 15-16, 1992.
- Ø 2. Riggs, J. A., Van Alstine, J. M., Schuman, T. A., Burns, N. L., and Harris, J. M. 1993. Comparison of Polysaccharide and PEG Coatings for Reduction of Protein Adsorption. Published in Proceedings of the 25th International Biomaterials Symposium, Birmingham, AL, April 28 - May 2, 1993.
- Ø 3. Van Alstine, J. M., Burns, N. L., Atharale, S., Holmberg, K and Harris, J. M. 1993. Use of Micro Particle Electrophoresis to Characterize Hydrophilic Polymer Coatings. American Chemical Society National Meeting, Chicago, IL, August 1993, Published as ACS Abstract .
- Ø 4. Emoto, K. E., Harris, J. M., Burns, N. L., Gundy, L. E. and Van Alstine, J. M. 1994. Electrophoretic Characterization of Poly(Ethylene Glycol) Coated Quartz Capillaries. ACS National Meeting, Birmingham, Alabama, October 1994.
- Ø 5. Gundy, L. E., Emoto, K. E., Burns, N. L., Harris, J. M. and Van Alstine, J. M. 1994. Polymer Coatings for Use in Separation Science. ACS National Meeting, Birmingham, Alabama, October 1994.

Invited Presentations

- Ø 1. Polymer Coatings to Control Surface Phenomena in Biotechnology. Department of Biomedical Engineering, University of Alabama, Birmingham, AL, October 20, 1993.
- Ø 2. Use of Microparticle Electrophoresis to Characterize Hydrophilic Polymer Coatings. Center for Surface Chemistry, University of Lund, Sweden, May 18, 1994.
- Ø 3. Polymer Coatings to Inhibit Complement Surface Activation. Institute for Surface Chemistry, Stockholm, Sweden, August 16, 1994.

Abstracts - 1994 ACS Meeting Birmingham

ELECTROPHORETIC CHARACTERIZATION OF POLY (ETHYLENE GLYCOL) COATED QUARTZ CAPILLARIES

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Microparticle electrophoresis and mathematical modeling are being used to evaluate poly(ethylene glycol) (PEG) coatings of biotechnical significance. PEG-epoxide (E-PEG) and PEG succinimidyl carbonate (SC-PEG) were grafted onto organosilane activated quartz surfaces. Coating variables studied include polymer MW, reaction conditions (pH, solvent, temperature), organosilane (mercapto- and amino-propylsilane), and coating longevity. E-PEG and SC-PEG yield similar coatings which effectively mask quartz surface charge at low ionic strength from pH 2 to 11. Differences in E-PEG and SC-PEG chemistry allowed testing and verification of an original site dissociation model of surface charge. The model, based on a Gouy-Chapman treatment of the electrical double layer, provides useful information related to coating surface density, effective thickness, and chemistry.

POLYMER COATINGS FOR USE IN SEPARATION SCIENCE

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Neutral hydrophilic polymer coatings are being evaluated for their ability to control interfacial phenomena which compromise the effectiveness of electrophoretic and other bioseparation methodologies on earth and in space. The phenomena in question include phase wall wetting, nonspecific adsorption, and electroosmosis. Variables studied include surface type (quartz vs plastic), surface activation, polymer type (polysaccharide vs polyether), and grafting chemistry. Electroosmosis, measured in a rapid, nondestructive manner via microparticle electrophoresis, has been found to correlate with polymer grafting density, longevity, chemistry and MW. Such measurements also correlate with the ability of polymer coatings to control phase wall wetting, nonspecific adsorption and particle electrophoretic mobility. The technique is therefore able to interface well with more common methods of surface analysis.

APPENDIX III

PUBLICATIONS AND PRESENTATIONS RELATED TO NASA GRANT NAS8-36955, D. O. 100

Publications

- (1) Boyce, J. F., Hovanes, B. A., Harris, J. M., Van Alstine, J. M. and Brooks, D. E. 1992. The Wetting Behavior of Aqueous Two-Phase Polymer Test Systems on Dextran Coated Glass Surfaces: Effect of Polymer Molecular Weight. J. Colloid Interf. Sci., 149 (1992) 153-161.
- (2) Brooks, D. E., Van Alstine, J. M., Stocks, S. J. and Sharp, K. A. 1993. PEG Derivatized Ligands with Hydrophobic and Immunological Specificity in Biomedical Applications of Polyethylene Glycol Chemistry, J. M. Harris, Ed., Plenum Press, New York.
- (3) Van Alstine, J. M., Burns, N. L., Riggs, J. A., Holmberg, K., and Harris, J. M. 1992. Electrokinetic Characterization of Polysaccharide Coatings, in Industrial Polysaccharide Chemistry, M. Yalpani Ed., ACS Symposia Series, American Chemical Society, 1992.
- (4) Van Alstine, J. M., Burns, N. L., Riggs, J. A., Holmberg, K., and Harris, J. M. 1992. Electrokinetic Characterization of Polymer Coatings. Colloids and Surfaces, submitted.
- (5) Bergström K., Holmberg, K., Riggs, J. A., Van Alstine, J. M., Burns, N. L., Shuman, T. P. and Harris, J. M. 1992. Protein Rejection By Polymer Modified Surfaces. Colloids and Surfaces, submitted.

Abstracts

- (6) Burns, N.L., Riggs, J. A., Harris, J. M. and Van Alstine, J. M. 1991. Electrokinetic Characterization of Polymer Coatings. 5th Annual Alabama Materials Research Conference., Birmingham, Alabama, September 26-27, 1991.
- (7) Riggs, J. A., Burns, N. L., Shuman, T. P., Harris, J. M. and Van Alstine, J. M. 1991. Electrokinetic Detection of Biomedically Important Surface Phenomena. 5th Annual Alabama Materials Research Conference., Birmingham, Alabama, September 26-27.
- (8) Burns, N.L., Riggs, J. A., Harris, J. M. and Van Alstine, J. M. 1991. Electrokinetic Characterization of Polymer Coatings. American Chemical Society, Southeast Regional Meeting, Richmond, V. A., November 13-15. Published in the abstracts.
- (9) Riggs, J. A., Burns, N. L., Shuman, T. P., Harris, J. M. and Van Alstine, J. M. 1991. Electrokinetic Detection of Biomedically Important Surface Phenomena. American Chemical Society, Southeast Regional Meeting, Richmond, V. A., November 13-15. Published in the abstracts.
- (10) Van Alstine, J. M., Burns, N. L., Riggs, J. A., Holmberg, K. and Harris, J. M. 1992. Characterization of Polysaccharide Coatings. American Chemical Society, National Meeting, Washington, D. C., August 23-29. Published in the abstracts.
- (11) Burns, N. L., Van Alstine, J. M. and Harris, J. M. 1992. Electrokinetic Evaluation of Organosilanes on Quartz. American Chemical Society, National Meeting, Washington, D. C., August 23-29. Published in the abstracts

(12) Van Alstine, J. M., Burns, N. L., Riggs, J. A., Holmberg, K. and Harris, J. M. 1992. Characterization of Polymer Coatings. International Meeting on Surface Modification - With Special Relevance to Biotechnology, Stockholm, August 19-21. Published in the abstracts.

(13) Harris, J. M., Van Alstine, J. M., Burns, N. L., Riggs, J. A., Osterberg, E., Shuman, T. P., Bergstrom, K. and Holmberg, K. 1992. Protein Rejection by Polysaccharide Modified Surfaces. International Meeting on Surface Modification - With Special Relevance to Biotechnology, Stockholm, August 19-21. Published in the abstracts.

(14) Harris, J. M., Van Alstine, J. M., Burns, N. L., Riggs, J. A., Shuman, T. P. and Holmberg, K.. 1992. Protein Rejection by Polysaccharide Modified Surfaces. 6th Annual Alabama Materials Research Conference, Auburn, October 15-16.

Presentations

(15) Van Alstine, J. M. 1990. Space Experiments in Aid of Bioseparations Technology, Beckman Instruments, Palo Alto, October 19.

(16) Van Alstine, J. M. 1991. Something Old, Something New, Something Borrowed, Something Blue - Current Bioseparations Research. Department of Biotechnology, Royal Institute of Technology, Stockholm, October 18.

(17) Van Alstine, J. M. 1992. Two New Bioseparations Methods. Center for Cell Research, Penn. State University, College Park, P. A., January 15.

(18) Van Alstine, J. M. 1992. Three New Bioseparations Techniques. Naval Research Laboratory, Washington, D. C., March 27.

(19) Van Alstine, J. M. 1992. Recent Bioseparations Research. Systemix Corporation, Palo Alto, July 6.